

Atty. Dkt. No. 065691-0216

B4
CONT. — homologous recombination using the EcoRI and XhoI restriction sites. The constructs were checked by restriction enzyme analysis.

REMARKS

The specification has been amended to include sequence identification numbers.
No new matter has been added.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE**Marked up rewritten paragraphs:**

On page 4, 2nd and 3rd paragraphs:

The peptides according to the present invention are the following:

01-P1 IQRTPKIQVYSRHPA (SEQ ID NO: 1)

(Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr-Ser-Arg-His-Pro-Ala)

02-P4 FHPSDIEVDLLKDGE (SEQ ID NO: 2)

(Phe-His-Pro-Ser-Asp-Ile-Glu-Val-Asp-Leu-Leu-Lys-Asp-Gly-Glu)

03-P9 ACRVNHVTL SQPKIV (SEQ ID NO: 3)

(Ala-Cys-Arg-Val-Asn-His-Val-Thr-Leu-Ser-Gln-Pro-Lys-Ile-Val)

It is also possible to use a smaller part (7 amino acids) of these 15 amino acids which lifts the neutralization of the virus by the monoclonal antibodies B1G6 or B2G2.2:

04-R-7-V RTPKIQV (SEQ ID NO: 4) (Arg-Thr-Pro-Lys-Ile-Gln-Val)

05-S-7-K SQPKIVK (SEQ ID NO: 5) (Ser-Gln-Pro-Lys-Ile-Val-Lys)

06-F-7-E FHPSDIE (SEQ ID NO: 6) (Phe-His-Pro-Ser-Asp-Ile-Glu)

On pages 4 and 5, bridging paragraph:

A common structure PKI (3 amino acids) appears to be the unit which is responsible; hence the following amino acid modifications:

07-TLSRTPKIQV (SEQ ID NO: 7) (Thr-Leu-Ser-Arg-Thr-Pro-Lys-Ile-Gln-Val) No. 185

08-IYLTQPKIKV (SEQ ID NO: 8) (Ile-Tyr-Leu-Thr-Gln-Pro-Lys-Ile-Lys-Val) No. 186

09-IQRTPKIQVY (SEQ ID NO: 9) (Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr) No. 187

10-TLSQPKIVKN (SEQ ID NO: 10) (Thr-Leu-Ser-Gln-Pro-Lys-Ile-Val-Lys-Asn) No. 188

11-IQRT PQIVKW (SEQ ID NO: 11) (Ile-Gln-Arg-Thr-Pro-Gln-Ile-Val-Lys-Trp) No. 189

12-IQRTPNIVKW (SEQ ID NO: 12) (Ile-Gln-Arg-Thr-Pro-Asn-Ile-Val-Lys-Trp) No.
190

On page 5, 1st, 2nd and 3rd full paragraphs:

It is also possible to introduce a cysteine and a glycosylation site:

13-CYNPSDIE (SEQ ID NO: 13) (Cys-Tyr-Asn-Pro-Ser-Asp-Ile-Glu)

14-YCNPEST (SEQ ID NO: 14) (Tyr-Cys-Asn-Pro-Glu-Ser-Thr)

15-NFLNCYVS (SEQ ID NO: 15) (Asn-Phe-Leu-Asn-Cys-Tyr-Val-Ser)

16-LNCYVSPSD (SEQ ID NO: 16) (Leu-Asn-Cys-Tyr-Val-Ser-Pro-Ser-Asp)

Finally, it is possible to use the peptides using the different variations according to the species (mice, primates, rabbits, guinea pigs):

17-KTPQIQV (SEQ ID NO: 17) (Lys-Thr-Pro-Gln-Ile--Gln-Val)

18-FHPPQIE (SEQ ID NO: 18) (Phe-His-Pro-Pro-Gln-Ile-Glu)

19-FHPPHIE (SEQ ID NO: 19) (Phe-His-Pro-Pro-His-Ile-Glu)

20-AEPKTVY (SEQ ID NO: 20) (Ala-Glu-Pro-Lys-Thr-Val-Tyr)

21-SQPKTVY (SEQ ID NO: 21) (Ser-Gln-Pro-Lys-Thr-Val-Tyr)

22-ILSRTPKIQV (SEQ ID NO: 22) (Ile-Leu-Ser-Arg-Thr-Pro-Lys-Ile-Gln-Val)

These peptides of SEQ ID NOS. 1 to 22 contain only the preferential choice; it is possible, as has been indicated above, to find equivalent peptides.

On page 6, 2nd full paragraph:

Analysis of the structure of the regions selected for P1, P9 and P10 can be carried out by methods such as the selection using alanine to replace each amino acid separately, particularly in the RTPKIQV (SEQ ID NO: 4) region, in order to determine the possible amino acids. It is also possible to use techniques using biotinylation of each peptide, followed by selection by EIA with the antibodies in order to determine the loss of attachment.

On page 13, 3rd full paragraph:

The peptide R7V (RTPKIQV) (SEQ ID NO: 4) was extended by 2 amino acids in order to allow the coupling. The structure used as immunogen is RTPKIQVGY (SEQ ID NO: 23).

On pages 15-16, bridging paragraph:

Methods

Chimeric recombinant viruses were constructed by PCR-directed mutagenesis. Two constructs based on the R7V sequence and HIV-1 LAV were obtained, in which seven amino acids of the V3 region of gp120 have been replaced by the R7V sequence. The positions of the mutated sequences are shown in the following table: (SEQ ID NOS 24, 4 and 4, respectively in order of appearance).

HIV-1 LAV (V3)	NNNTRKSIRIQRGPGRFVT		
R7V	RTPKIQV	(1)	RPL
R7V	RTPKIQV	(2)	PLG

The EcoRI₅₂₋₇₈-XhoI₈₄₀₁ fragment of HIV-1 LAV cloned into the vector Bluescript was used as template for subsequent constructs. In the first stage, the DNA fragments flanked by primers containing the BglII restriction site at one end and the nucleotide sequence encoding R7V at the other end were synthesized for the RPL and PLG constructs by PCR amplification. The mutagenesis oligonucleotides used consisted of a (+) primer ACACCAAAGATACAAGTTGTTACAAATAGGAAAA (SEQ ID NO: 25) and a (-) primer TTGTATCTTTGGTGTCTCTGGATCCGGATACTTT (SEQ ID NO: 26) for the RPL construct and of a (+) primer CGTACACCAAAAATCCAGGTCCAGAGAGGACCA (SEQ ID NO: 27) and a (-) primer GATTTTTGGTGTACGCGTATTGTTGTTGGGTCT (SEQ ID NO: 28) for the PLG construct. In the second stage, two PCR products for each construct were mixed and amplified using the primers containing the BglII restriction sites. The RPL and PLG fragments were cleaved by the enzyme BglII and inserted into the vector Bluescript containing the EcoRI₅₂₇₈-XhoI₈₄₀₁ fragment of HIV-1 LAV, cleaved by BglII. In addition to the R7V sequence, the amplification primers contained modifications in the nucleotide sequence leading to the appearance of new BamHI and MluI restriction sites in the RPL and PLG constructs respectively, without additional modifications in the amino acid sequence. The new restriction sites were used to

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screen the mutated sequences. Finally, the EcoRI₅₂₇₈-XhoI₈₄₀₁ fragments of HIV-1 LAV containing the RPL and PLG constructs were inserted into the plasmid pNL4-3 by homologous recombination using the EcoRI and XhoI restriction sites. The constructs were checked by restriction enzyme analysis.